

Research



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Evidence for atypical nest overwintering by hatchling lizards, *Heloderma suspectum*

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The timing of reproductive events (e.g. oviposition and hatching) to coincide with favourable seasonal conditions is critical for successful reproduction. However, developmental time may not match the duration between the optimal time for oviposition and the optimal time for hatchling survival. Thus, strategies that alter the time between oviposition and hatchling emergence can be highly advantageous. Arrested development and the resulting extension of the duration between oviposition and hatching has been widely documented across oviparous amniotes, but nest overwintering by hatchlings has only been documented in aquatic chelonians that live where winters are quite cold. Herein, we present a compilation of evidence regarding reproductive phenology by hatchlings of the Gila monster (*Heloderma suspectum*), a lizard inhabiting the Sonoran Desert of North America. Our data demonstrate that (i) Gila monster hatchlings from eggs oviposited in July do not emerge from their nests until late spring or summer of the following year, yet (ii) Gila monster eggs artificially incubated at field-relevant temperatures hatch in 4–5 months. Furthermore, we describe a fortuitous excavation of a hatching Gila monster nest in late October, which coincides with the artificial incubation results. Together, these results provide strong support for the existence of overwintering in the nest by a lizard, and suggest that this reproductive strategy should be explored in a broader array of taxa.

1. Introduction

The timing of reproduction in oviparous species is under selective pressure to have oviposition and offspring emergence occur at times when conditions are advantageous [1,2]. As development and survival are highly influenced by external conditions such as temperature and moisture [3–6], predator presence [7] and tidal schedule [8], oviposition should occur when such conditions are optimal. Similarly, offspring should hatch from eggs or emerge from the nest in alignment with specific environmental cues [9], for example, when energy and water resources are plentiful [10,11] or when hatching or emergence enables avoidance of predators [12,13] or pathogens [14]. However, environmental conditions can be unpredictable, so it may be impossible to predetermine the optimal timing of oviposition and hatching or emergence [15,16]. Therefore, many species have evolved reactive strategies that delay the time of emergence until environmental conditions are favourable for neonates [17].

Delays in the emergence of hatchlings are often a result of arrested embryonic development at the time of oviposition (i.e. embryonic diapause) or during the final stage of development (i.e. delayed hatching), and such delays enable better alignment of hatching with specific environmental conditions [18,19]. Some form of arrested development is known to occur in all major extant groups of egg-laying vertebrates [20–22].

An alternative to arresting embryonic development to match offspring presence with favourable environmental conditions is to, instead, delay emergence from the nest by hatchlings. In such cases, it may be adaptive to hatch before conditions are optimal, but delay emergence from the nest until the hatchlings detect cues that indicate when conditions improve. Short-term delays (hours to days) in emergence are relatively common. For example, Greek tortoises, *Testudo graeca*, remain in the nest after hatching for 1–23 days, presumably to absorb the remaining content of the externalized yolk sac [23]. Green iguanas (*Iguana iguana*) hatch asynchronously but wait to emerge in groups to reduce predation risk [24]. Sea turtle hatchlings often remain in the nest until synchronously emerging at night or on rainy days to avoid excessive heat [25–27] and reduce predation risk [28].

Unlike short-term delays, extended (weeks to months) delays in emergence by hatchlings are uncommon, having been reported only within aquatic chelonians, especially but not exclusively where winters are cold [29,30]. In these cases, the offspring hatch from their eggs but overwinter in the terrestrial nest until temperatures in spring are conducive to activity.

Interestingly, to date there are no reported instances of extended-duration nest overwintering by hatchlings of a terrestrial species regardless of taxon, or of species in environments where winter temperatures are quite mild (i.e. no risk of freezing temperatures throughout winter). Thus, our understanding is driven by aquatic turtles, in which extended delays in nest emergence are thought to be evolutionarily driven by the fact that hatchlings have relatively high mass-specific metabolic rates and thus are intolerant of the chronically hypoxic conditions found underwater [31]. As many adult aquatic turtles overwinter underwater to avoid freezing, hatchlings of these species require a different overwintering strategy that provides greater oxygen availability [31]. However, nest overwintering is not ubiquitous among aquatic turtles living in cold climates, with variation even occurring among sympatric species [30] and within a species [32].

While inconsistencies in the use of nest overwintering among cold-winter aquatic turtles have drawn considerable attention, a topic of equal interest is whether nest overwintering is employed by other species facing different environmental constraints, such as in terrestrial species. Accordingly, herein we examine an apparent disassociation between incubation duration and hatchling emergence in the Gila monster (*Heloderma suspectum*), a relatively large lizard from the Sonoran Desert in North America.

Gila monsters typically lay eggs in July, coinciding with the onset of the summer monsoon season [33]. Under laboratory conditions, incubation lasts 114–152 days, with the duration influenced by temperature. However, neonate Gila monsters are not seen in the wild until late April at the earliest and, more typically, May through to early August (9–12 months after oviposition) [33]. This discrepancy between expected incubation duration and the observation of neonates led Beck [33] to question whether eggs in the wild have an extended developmental time as a result of decreasing temperatures during the autumn and winter. Alternatively, he proposed that eggs may hatch in the autumn, after a duration similar to that seen in captivity, but hatchlings overwinter in the nest. As an additional possibility,

hatchlings may emerge from the nest in autumn, but remain secretive or search out a different refuge in which to spend the winter. We present (i) data on the timing of emergence of offspring from two naturally laid Gila monster clutches, (ii) results from a laboratory experiment in which Gila monster eggs were incubated using a thermal profile based on sub-surface temperatures in the field, and (iii) details from an opportunistic excavation of a hatching Gila monster nest. Together, these observations provide strong support for a strategy of delayed emergence owing to nest overwintering by hatchling Gila monsters.

2. Material and methods

(a) Study species and its natural environment

Gila monsters are the largest lizard (30–33 cm snout–vent) in the USA and a member of the only lizard genera that is widely accepted as venomous. Gila monsters predominantly reside in the Arid Upland subdivision of the Sonoran Desert. Climate in this region is dramatically seasonal. Air temperatures are cool (maximum and minimum air temperatures: 10–20°C and 0–10°C, respectively) in winter, mild (maximum and minimum air temperatures: 15–30°C and 5–20°C, respectively) in early spring (mid-March to April), and hot (maximum and minimum air temperatures: 28–40°C and 10–25°C, respectively) during late spring and summer (May to September) [34]. Rainfall is biphasic with about half the rain falling relatively lightly during the winter and early spring and half the rain falling in the form of relatively intense thunderstorms during late summer (mid-July to mid-September). These two rainy periods are separated by a hot, dry period that typically lasts 14–16 weeks.

Gila monsters forage almost exclusively on the contents of vertebrate nests—nestling rodents and rabbits, as well as bird, tortoise and lizard eggs [33]. Given this limited diet and the harsh conditions of the desert, food availability is highly seasonal, with mammal and bird nests available mostly in early spring and to a lesser extent during the summer rainy season, while reptile eggs are mostly available in late spring and early summer.

Gila monsters are capital breeders, relying on energy stores to produce clutches, and, as a result, typically reproduce only every 2–4 years [33]. To survive intermittent availability of food and water as well as to accumulate sufficient resources for reproduction, a Gila monster stores considerable amounts of fat in its tail [34] and uses its urinary bladder as an internal water reservoir [35].

(b) Timing of hatchling emergence

To determine the timing of oviposition and nest emergence in Gila monsters, from 2001 to 2012 we monitored radio-telemetered Gila monsters at a long-term field site where they were being used for other studies (see [36] for a description of the implantation procedure). Reproductive status of females was determined via ultrasonography (Concept/MLV, Dynamic Imaging, Livingston, Scotland), and gravid females nearing their oviposition dates were tracked on a near-daily basis. A refuge site was suspected as a nesting site when a gravid female emerged from the burrow looking considerably thinner than she did prior to entering days earlier. We then used a spring scale (Pesola no. 93053, Schindellegi, Switzerland) to verify that there had been a substantial (>150 g) reduction in body mass since her last recorded mass.

Owing to the inherent difficulty in finding Gila monsters active above ground during the dry summer, visual assessments of the Gila monsters were often separated by a week or more.

This, along with the low frequency of reproduction of females, made successful identification of nest sites difficult. Six different burrow systems (four of white-throated woodrats, *Neotoma albigula*; two of Merriam's kangaroo rats, *Dipodomys merriami*) were suspected as nest sites, and we surrounded all detected entrances of each of these burrow systems with a ring of aluminium flashing and buried pitfall traps at 3–5 locations along the inner perimeter of the ring.

Pitfall traps consisted of 191 plastic buckets containing a small plastic container that was filled with moist substrate and had an access hole cut into the side. A fine stick was placed in each trap to allow insects and small lizards, but not hatchling Gila monsters, to escape. The pitfall traps were mostly placed in the shade of a bush and were shaded with plywood and, frequently, also shade cloth. The placement of the traps, the artificial shade, and the moist container enabled trapped hatchling Gila monsters to survive for several days, but traps were typically checked every 1–2 days. When a Gila monster was found in a trap, we recorded the date and the Gila monster's mass (using a 100 g Pesola spring scale) and snout–vent length (using a rigid ruler). It was then released outside of the ring of aluminium flashing so it could freely disperse.

(c) Incubation experiment

Under laboratory conditions, Gila monster egg incubation lasts 114–152 days [33]. As incubation duration is highly influenced by temperature, it is possible that, in a natural nest, the cool temperatures of autumn and winter slow development or even induce developmental diapause, resulting in a much longer incubation period, with hatching in spring or early summer, 9–12 months after oviposition. To test this possibility, we estimated the temperature of a natural Gila monster nest over the course of a year and then incubated captive-produced eggs under this thermal regime.

We estimated the temperature profile of a natural Gila monster nest by hammering a piece of $\frac{1}{2}$ inch (1.25 cm) diameter rebar 1 m into the ground approximately 1 m from a burrow system in which we knew a Gila monster recently oviposited a clutch. We then replaced the rebar with a wooden dowel that had thermocouples attached at 15, 30, 60 and 90 cm below the ground surface. Temperatures from these thermocouples were recorded hourly (using a well-shaded 21x Micrologger, Campbell Scientific, Logan, UT, USA) from the time the female left the nest in July through to the emergence of hatchlings the next spring and early summer.

The female Gila monster that used this nest had, at the time of its radiotransmitter implantation, also been intracoelomically implanted with an approximately 3 g temperature logger (iButton Thermochron, Maxim Integrated, San Jose, CA, USA). By matching the female's thermal profile while in the nest with the data from the thermocouple array from those days, we estimated the depth of the nest (between 60 and 90 cm deep, as temperatures at those depths are nearly identical). We then used the 90 cm array temperature for the ensuing months as a thermal regimen for incubation of eggs produced by captive Gila monsters.

A balanced split-clutch design was used to divide four or six eggs from each of four clutches equally between two incubation treatments—a standard incubation temperature (control; constant $29.3 \pm 0.5^\circ\text{C}$; $n = 10$) and a thermal regime derived from the wild nest (experimental; maximum temperature 32.5°C , minimum temperature 17.8°C , average temperature 27.3°C from the beginning of incubation until the last hatchling emerged from its egg; $n = 10$; figure 1). Incubation temperature was controlled by placing the eggs in custom incubators with thermoelectric modules that use bi-directional controllers (FTC100, Ferrotec (USA) Corporation, Santa Clara, CA USA). During incubation,

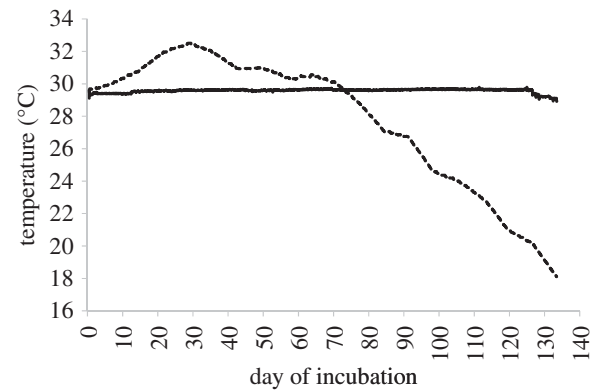


Figure 1. Thermal profiles used to incubate Gila monster eggs in captivity. The solid line represents the norm for optimal artificial incubation (control), while the dashed line is the temperature profile recorded from a nest in the wild.

the eggs in both treatment groups were placed on hydrated vermiculite ($0.5 \text{ g water g}^{-1}$ dry vermiculite) within plastic incubation containers, which is a well-established, effective incubation environment (M. Seward 2018, unpublished data). Eggs and neonates were weighed at the time of oviposition and emergence from the eggs, respectively. For each egg that successfully hatched, we determined incubation duration (i.e. days between oviposition and pipping) and the number of days between pipping and full emergence from the egg by the hatchling.

(d) Excavation of a hatching Gila monster nest

On 28 October 2016, R.R. was contacted by a construction contractor who was excavating a trench in preparation for remodelling a home in the Catalina Foothills near Tucson, AZ, which was 17 and 36 km southeast of the two nests from which we report emergence dates (all three nests are within the range of 850 to 940 m in elevation). The contractor reported that, during excavation, he had accidentally unearthed a Gila monster nest that contained hatching eggs. R.R. proceeded to the site where he collected the hatchlings and thoroughly searched for additional hatchlings. Two days after excavation, any hatchlings still within their pipped eggshells were carefully removed and all hatchlings were weighed and measured.

(e) Statistical analyses

We used emergence data from nest sites to test whether the mass of neonates decreases with later emergence dates, as an indicator of mass loss out of the egg over time. We calculated Pearson's correlation coefficients between day of emergence and neonate mass for each nest separately and for pooled nest data.

Data from the incubation experiment were used to test whether control and experimental groups differed in mass of eggs at oviposition, incubation duration, duration between pipping and hatching, or mass of hatchlings. Normality was visually assessed using Q–Q plots, and Levene's test was used to determine equality of variances. Egg mass and mass of hatchlings were normally distributed and control and experimental groups had equal variances, so we tested differences between groups with normal *t*-tests. Incubation duration had a non-normal distribution that transformations could not fix; thus, we tested differences using a Wilcoxon test. Duration between pipping and hatching had non-normal distributions and unequal variances that transformations could not correct; thus, we used a Welch's *t*-test to compare the results from the two thermal regime treatment groups. We also calculated Kendall's rank correlation coefficient to compare incubation duration with the time

Table 1. Date of emergence, snout–vent length (SVL), and mass of neonate *Gila* monsters from two natural nests.

ID	emergence date	SVL (mm)	mass (g)
nest 1			
1-1	4 May 2001	136	33.42
1-2	9 June 2001	137	26.95
1-3	26 June 2001	135	23.12
1-4	1 July 2001	141	23.72
1-5	4 Aug 2001	137	24.16
averages	20 June 2001	137	26.27
nest 2			
2-1	2 May 2010	125	32.23
2-2	19 May 2010	131	32.01
2-3	4 June 2010	134	37.39
2-4	4 June 2010	133	31.57
2-5	7 June 2010	127	28.79
2-6	9 June 2010	128	24.07
2-7	25 June 2010	130	20.32
2-8	4 July 2010	121	26.64
averages	5 June 2010	129	29.13

it took to hatch (i.e. duration between when hatchlings first pipped and when they left their eggs). All statistics were performed using R v. 3.0.3 [37].

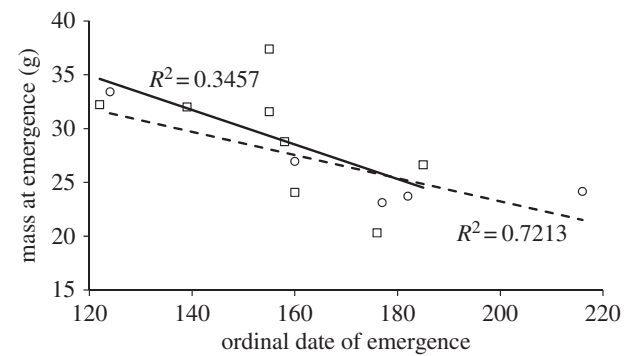
3. Results

(a) Timing of hatchling emergence

Four suspected nest sites failed to produce any hatchlings in the pitfall traps, probably as a result of either misidentification of a nest, predation, egg desiccation or failure to surround all refuge entrances within the ring of flashing. However, we collected emergence data for two nests.

Nest 1: a gravid female first entered this woodrat burrow system sometime between 17 June and 26 June 2001. On 9 July, she was still in the burrow, but, on 10 July, she was tracked to a burrow approximately 50 m away. Thus, the female spent 13–22 days in the suspected nest burrow system. On 12 July, she was found surface-active, captured and weighed, and she had lost 265 g since her last mass was taken on 13 May. Given the high likelihood that we identified her nest site, we surrounded the burrow system with a drift fence and pitfall traps. Between 4 May and 4 August 2002, five neonate *Gila* monsters were captured in the pitfall traps (table 1). Despite the small sample size, the relationship between neonate mass and emergence date approached but did not achieve a significant correlation ($t_3 = -2.7864$; $p = 0.0686$; figure 2).

Nest 2: while specific oviposition details are not available for the gravid female that used this nest (owing to missing field notes), she oviposited within this kangaroo rat burrow system in July of 2009, and, based on behaviour and change in mass that was similar to the female that laid in nest 1, we suspected that we identified her nest site. Between 2 May and 4 July 2010, eight neonate *Gila* monsters were

**Figure 2.** Emergence mass relative to date of emergence for nest 1 (open circles, dashed line) and nest 2 (open squares, solid line). When data for the two clutches were pooled, Pearson's correlation coefficient showed emergence mass decreased as hatchlings emerged later in the year ($t_{11} = -3.1146$; $p < 0.01$; correlation coefficient = -0.6846). See text for additional statistics.

captured in pitfall traps surrounding this nest (table 1). Neonate mass was not correlated with emergence date ($t_6 = -1.7803$; $p = 0.125$; figure 2).

However, when data from both nest 1 and nest 2 were pooled, the Pearson's correlation coefficient showed a significant negative correlation between neonate mass and emergence date ($t_{11} = -3.1146$; $p = 0.00984$; correlation coefficient = -0.6846 ; figure 2).

(b) Incubation experiment

All 10 control eggs hatched, while only 7 of the 10 experimental eggs hatched. Two of the three eggs that failed to hatch had fully developed *Gila* monsters inside, but egg and hatchling mass data from all three of these eggs were not included in the primary analyses. There was no significant difference in incubation duration between the control (mean = 126 ± 1 days, range 124–129 days) and experimental (mean 128 ± 1 days, range 124–133 days) groups ($W = 22.5$; $p = 0.234$). However, hatchlings in the experimental group took longer to exit the egg once they had pipped (control: 1.3 ± 0.2 days, range 1–2 days; experimental: 10.2 ± 0.9 days, range 7–14 days; $t_6 = -8.2346$; $p = 0.000128$), but pipping to emergence duration was not significantly related to incubation duration ($z = 1.7674$; $p = 0.0772$; figure 3). There were no differences between the two treatments in the mass of the eggs at oviposition (control: 34.90 ± 0.82 g; experimental: 35.12 ± 0.84 g; $t_{15} = -0.18783$; $p = 0.854$) and the mass of the hatchlings when they left the eggshell (control: 29.63 ± 0.92 g; experimental: 29.77 ± 0.88 g; $t_{15} = -0.10878$; $p = 0.915$). The relative results do not change if the three eggs that failed to hatch and the fully developed embryos found dead in two of these eggs were included in the treatment group dataset (eggs at oviposition: $p = 0.4007$; hatchling mass: $p = 0.469$).

(c) Excavation of a hatching *Gila* monster nest

Five neonates were found in the nest that was excavated on 18 October 2016. At the time of discovery, two of the neonates were fully out of their eggs, one was halfway out of its egg with its umbilicus still connected to the egg, and two had slit their eggshells multiple times but were fully within the egg (table 2). The excavation date and the variable stages of hatching among the clutch mates are consistent with the

Table 2. Hatching status, SVL and mass of hatchlings removed from excavated nest.

ID	hatching status when found	SVL (mm)	mass (g)
E-1	halfway out of the egg, umbilicus intact	123	33.47
E-2	fully hatched, dried umbilical stump	126	27.23
E-3	fully hatched, dried umbilical stump	124	27.57
E-4	pipped, umbilicus intact	126	32.38
E-5	pipped, umbilicus intact, umbilical hernia	128	23.31
averages		125	28.79

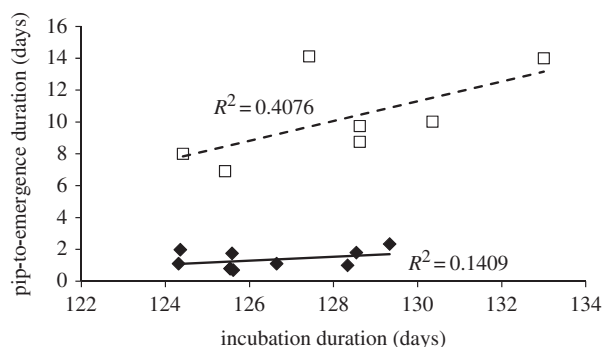


Figure 3. Incubation duration was similar for eggs incubated at a constant 29.3°C (black diamonds and solid line) and eggs incubated under a natural regimen (open squares and dashed line) ($p = 0.234$). However, Welch's t -tests showed hatchlings incubated under the natural thermal cycle took longer to exit the egg after pipping (control: 1.3 ± 0.2 days; experimental: 10.2 ± 0.9 days; $t_6 = -8.2346$; $p < 0.001$), and incubation duration did not affect the time to exit the egg after pipping ($p = 0.077$).

laboratory egg incubation using natural thermal conditions where hatchlings pipped 124–133 days after being laid and took 7–14 days to emerge from the egg. Combined, these observations provide strong evidence that Gila monster eggs in wild nests hatch in the autumn. At examination two days later, the three neonates still within the egg, either partially or fully, were removed for processing. Mean snout–vent length (SVL) and mass of the hatchlings were 125 mm and 28.79 g, respectively (table 2).

4. Discussion

While our low sample sizes do not enable us to state that all Gila monster hatchlings use this strategy, we are able to provide a compilation of evidence that supports nest overwintering by neonate Gila monsters. We demonstrate through artificial incubation and excavation of a natural nest at the time of hatching that Gila monster eggs hatch in the autumn. Observations made from pitfall traps around known nest sites show that neonates may not emerge from their nest sites until late spring through to mid-summer.

Until now, nest overwintering was known only for some aquatic turtles, and freezing winter temperatures seem to be important for the use of this strategy. In warm temperature regions, spring hatchling emergence in turtles, when it occurs, is typically a result of embryonic diapause, not hatchling overwintering [21,38]. However, where a freeze occurs in winter, turtles cannot use diapause because embryonic

turtles are unable to survive cold temperatures [39], thus forcing hatching before the onset of winter. While hatchlings can tolerate cold temperatures, they are intolerant of hypoxia, preventing aquatic hibernation, which is the means by which the adult turtles overwinter [31]. The importance of aquatic hibernation, and, therefore, hatchling hypoxia, in contributing to a species' use of nest overwintering is supported by the fact that the terrestrial eastern box turtle, *Terrapene carolina*, does not overwinter in its nest despite being confamilial and sympatric with many of the species in which nest overwintering has been documented. Instead of overwintering in the nest, eastern box turtles emerge in the autumn and overwinter buried underground 2–10 m from the nest [40].

The fact that few other species face this combination of constraints (embryonic cold intolerance and an adult overwintering strategy that puts them under extremely hypoxic conditions) could explain why nest overwintering had not been documented in other oviparous vertebrates. Accordingly, our evidence for nest overwintering in Gila monsters is intriguing for multiple reasons. First, it is, to our knowledge, the first documentation of nest overwintering by a squamate, a group of reptiles that have been separated from Chelonians for 200–250 million years [41]. More importantly, Gila monsters are neither aquatic nor from an environment where freezing temperatures are common during the winter. During the winter, the Sonoran Desert rarely experiences freezing air temperatures and, during the rare times that they do occur, they only last for several hours. Therefore, embryos could probably survive winter conditions. However, because they are venomous, hatchlings in a nest are probably better at defending themselves from predators than are eggs, which would be the situation if they used embryonic diapause to achieve spring emergence.

If emergent Gila monster hatchlings are not at risk of overwintering hypoxia or freezing, why do they choose not to emerge from the nest? Furthermore, unlike nest-overwintering neonate turtles, why do Gila monster neonates hold off emergence until mid-spring through to mid-summer, months after weather conditions are favourable for activity [33]? In fact, the weather is much more conducive to activity in early spring than it is during the mid-spring or early summer, when many hatchling Gila monsters emerge; in summer months before monsoon season, daytime high temperatures typically are 40–45°C and, during most years, rainfall is non-existent. Emerging when it is extremely hot and dry is physiologically very demanding, so, intuitively, seems like a poor strategy. Adult Gila monsters survive hot, dry periods by greatly decreasing surface activity [33,34,36],

yet most hatchlings begin their surface activity under these conditions. In the closely related Mexican bearded lizards (*Heloderma horridum*), egg laying is delayed until December, and incubation duration (based on captive breeding) is much longer than that of Gila monsters and results in hatching and prompt emergence occurring at the onset of the summer rains in June or July, a period of high water availability and community productivity [33]. The differences between these two closely related species in both how they extend the time between oviposition and emergence as well as the timing of emergence relative to rainfall patterns may provide a good opportunity to better understand reproductive phenology and the strategies employed to achieve the timing of events. Thus, further work comparing reproduction in these two species would be insightful.

One possible explanation for the emergence pattern we document for hatchling Gila monsters is that emergence is driven primarily by food availability. That is, why come to the surface if there is little to no chance of finding food? As nest raiders, Gila monsters only feed when other vertebrates are producing offspring. Eggs and young vertebrate prey are not available in autumn or winter in the Sonoran Desert, so even adult Gila monsters greatly limit activity during these times [33], mostly only surfacing to move to overwintering sites or to bask near the entrance of the refuge (D.F. DeNardo 2000–2012, unpublished data). The first half of spring is the main breeding season for most birds and small mammals, and, as such, it is a period of high activity and weight gain for adult Gila monsters [36]. However, the contents of these nests are mostly too large for hatchling Gila monsters to consume. The eggs of small lizards are the predominant prey of hatchling Gila monsters, and hatchling Gila monster emergence occurs when these small lizards are the most productive [33]. Thus, emerging earlier but searching out a different refuge would probably only result in an energetic cost, rather than producing any energetic benefit. Additionally, though our sample size is small, our drift fence with pitfall results suggest hatchlings did not attempt to leave the nest earlier in the year to find alternative refugia, making it unlikely that the secretive nature of neonates leads to failure to detect actual movements shortly after hatching.

Another possible influence on the late emergence of hatchling Gila monsters is night-time temperature. Adult Gila monsters are typically diurnal during the first half of spring and switch to mostly nocturnal activity around mid-May when daytime temperatures get too warm but night

temperatures are conducive for activity by an ectotherm [33]. The vast majority of observations of hatchling Gila monsters in the wild, whether opportunistic or via radio-tracking, are at night (D.F. DeNardo 2000–2012, unpublished data). Nocturnal activity by hatchlings might reduce predation risk and, therefore, drive emergence time, or, alternatively, the timing of emergence might force hatchling Gila monsters to be nocturnal. Differentiating these contrasting possibilities would require more extensive studies, including artificially manipulating emergence date.

While it would be valuable to further explore what drives hatchling Gila monsters to overwinter in the nest and delay emergence until late spring, it would be equally rewarding to expand the exploration for additional species where nest overwintering might occur. It is possible that nest overwintering is more widespread, but simply underexplored. With the identification of taxonomically and ecologically diverse instances of hatchling overwintering in the nest, we will be able to produce a more comprehensive list of traits that favour nest overwintering and thus a better understanding of the evolutionary significance of this reproductive strategy.

Ethics. All described fieldwork was conducted under permits from the Arizona Game and Fish Department and protocols from the Arizona State University Institutional Animal Care and Use Committee (05-783R, 08-962R, 09-1044R, 12-1244R, and 15-1410R). The captive incubation study was performed in Colorado, where no permits are required for possessing Gila monsters.

Data accessibility. Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.s1r15c6> [42].

Authors' contributions. D.D. and K.M. conceived of the study, performed fieldwork and drafted the manuscript. M.S. collected the laboratory-derived data and provided comments to the manuscript. R.R. collected field data and provided comments to the manuscript. All authors gave final approval for publication.

Competing interests. The authors have no competing interests.

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References

1. Van Noordwijk AJ, McCleery RH, Perrins CM. 1995 Selection for the timing of great tit breeding in relation to caterpillar growth and temperature. *J. Anim. Ecol.* **64**, 451–458. (doi:10.2307/5648)
2. Love OP, Gilchrist HG, Descamps S, Semeniuk CAD, Bêty J. 2010 Pre-laying climatic cues can time reproduction to optimally match offspring hatching and ice conditions in an Arctic marine bird. *Oecologia* **164**, 277–286. (doi:10.1007/s00442-010-1678-1)
3. Gutzke WH, Packard GC. 1987 Influence of the hydric and thermal environments on eggs and hatchlings of bull snakes *Pituophis melanoleucus*. *Physiol. Zool.* **60**, 9–17. (doi:10.1086/physzool.60.1.30158624)
4. Clark BR, Faeth SH. 1998 The evolution of egg clustering in butterflies: a test of the egg desiccation hypothesis. *Evol. Ecol.* **12**, 543–552. (doi:10.1023/A:1006504725592)
5. Gamboa MI. 2005 Effects of temperature and humidity on the development of eggs of *Toxocara canis* under laboratory conditions. *J. Helminthol.* **79**, 327–331. (doi:10.1079/JOH2005287)
6. Sutherst RW, Bourne AS. 2006 The effect of desiccation and low temperature on the viability of eggs and emerging larvae of the tick, *Rhipicephalus (Boophilus) microplus* (Canestrini) (Ixodidae). *Int. J. Parasitol.* **36**, 193–200. (doi:10.1016/j.ijpara.2005.09.007)
7. Johnson JB, Saenz D, Adams CK, Conner RN. 2003 The influence of predator threat on the timing of a life-history switch point: predator-induced hatching

- in the southern leopard frog (*Rana sphenoccephala*). *Can. J. Zool.* **81**, 1608–1613. (doi:10.1139/z03-148)
8. Christy J.H. 2011 Timing of hatching and release of larvae by Brachyuran crabs: patterns, adaptive significance and control. *Integr. Comp. Biol.* **51**, 62–72. (doi:10.1093/icb/icr013)
 9. Doody JS. 2011 Environmentally cued hatching in reptiles. *Integr. Comp. Biol.* **51**, 49–61. (doi:10.1093/icb/icr043)
 10. Joosse ENG, Testerink GJ. 1977 The role of food in the population dynamics of *O. cincta* (Linne) (Collembola). *Oecologia* **29**, 189–204. (doi:10.1007/BF00345694)
 11. Warner DA, Shine R. 2007 Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia* **154**, 65–73. (doi:10.1007/s00442-007-0809-9)
 12. Warkentin KM. 2000 Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. *Anim. Behav.* **60**, 503–510. (doi:10.1006/anbe.2000.1508)
 13. Chivers DP, Kiesecker JM, Marco A, Devito J, Anderson MT, Blaustein AR. 2001 Predator-induced life history changes in amphibians: egg predation induces hatching. *Oikos* **92**, 135–142. (doi:10.1034/j.1600-0706.2001.920116.x)
 14. Warkentin KM, Currie CR, Rehner SA. 2001 Egg-killing fungus induces early hatching of red-eyed treefrog eggs. *Ecology* **82**, 2860–2869. (doi:10.1890/0012-9658(2001)082[2860:EKFIEH]2.0.CO;2)
 15. Perrins CM. 1970 The timing of birds' breeding seasons. *Ibis* **112**, 242–255. (doi:10.1111/j.1474-919X.1970.tb00096.x)
 16. Verhulst S, Nilsson J. 2008 The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. *Phil. Trans. R. Soc. B* **363**, 399–410. (doi:10.1098/rstb.2007.2146)
 17. Shine R, Brown GP. 2008 Adapting to the unpredictable: reproductive biology of vertebrates in the Australian wet-dry tropics. *Phil. Trans. R. Soc. B* **363**, 363–373. (doi:10.1098/rstb.2007.2144)
 18. Andrews RM, Donoghue S. 2004 Effects of temperature and moisture on embryonic diapause of the veiled chameleon (*Chamaeleo calytratus*). *J. Environ. Zool.* **301A**, 629–635. (doi:10.1002/jez.a.56)
 19. Morrison FM, Harlow PS, Keogh JS. 2009. Nesting ecology of the critically endangered Fijian crested iguana *Brachylophys vitiensis* in a Pacific tropical dry forest. *Pac. Conserv. Biol.* **15**, 135–147. (doi:10.1071/PC090135)
 20. Ewert MA. 1991 Cold torpor, diapause, delayed hatching and aestivation in reptiles and birds. In *Egg incubation: its effects on embryonic development in birds and reptiles* (eds DC Deeming, MWJ Ferguson), pp. 173–191. Cambridge, UK: Cambridge University Press.
 21. Rafferty AR, Reina RD. 2012 Arrested embryonic development: a review of strategies to delay hatching in egg-laying reptiles. *Proc. R. Soc. B* **279**, 2299–2308. (doi:10.1098/rspb.2012.0100)
 22. Morrow GE, Jones SM, Nicol SC. 2015 Frozen embryos? Torpor during pregnancy in the Tasmanian short-beaked echidna *Tachyglossus aculeatus* setosus. *Gen. Comp. Endocrinol.* **244**, 139–145. (doi:10.1016/j.ygcen.2015.11.006)
 23. Diaz-Paniagua C, Keller C, Andreu AC. 1997 Hatching success, delay of emergence and hatchling biometry of the spur-thighed tortoise, *Testudo graeca*, in south-western Spain. *J. Zool. Lond.* **243**, 543–553. (doi:10.1111/j.1469-7998.1997.tb02800.x)
 24. Burghardt GM, Greene HW, Rand AS. 1977 Social behavior in hatchling green iguanas: life at a reptile rookery. *Science* **195**, 689–691. (doi:10.1126/science.195.4279.689)
 25. Mrosovsky N. 1968 Nocturnal emergence of hatchling sea turtles: control by thermal inhibition of activity. *Nature* **220**, 1338–1339. (doi:10.1038/2201338a0)
 26. Drake DL, Spotila JR. 2001 Thermal tolerances and the timing of sea turtle hatchling emergence. *J. Therm. Biol.* **27**, 71–81. (doi:10.1016/S0306-4565(01)00017-1)
 27. Glen F, Broderick AC, Godley BJ, Hays GC. 2006 Thermal control of hatchling emergence patterns in marine turtles. *J. Exp. Mar. Biol. Ecol.* **334**, 31–42. (doi:10.1016/j.jembe.2006.01.005)
 28. Santos RG, Pinheiro HT, Martins AS, Riul P, Bruno SC, Janzen FJ, Ioannou CC. 2016 The anti-predator role of within-nest emergence synchrony in sea turtle hatchlings. *Proc. R. Soc. B* **283**, 20160697. (doi:10.1098/rspb.2016.0697)
 29. Gibbons JW, Nelson DH. 1978 The evolutionary significance of delayed emergence from the nest by hatchling turtles. *Evolution* **32**, 297–303. (doi:10.1111/j.1558-5646.1978.tb00645.x)
 30. Baker PJ, Costanzo JP, Iverson JB, Lee Jr RE. 2013 Seasonality and interspecific and intraspecific asynchrony in emergence from the nest by hatchling freshwater turtles. *Can. J. Zool.* **91**, 451–461. (doi:10.1139/cjz-2012-0335)
 31. Costanzo JP, Lee Jr RE, Ultsch GR. 2008 Physiological ecology of overwintering in hatchling turtles. *J. Exp. Zool. A Ecol. Genet. Physiol.* **309A**, 297–379. (doi:10.1002/jez.460)
 32. Riley JL, Tattersall GJ, Litzgus JD. 2014 Potential sources of intra-population variation in the overwintering strategy of painted turtle (*Chrysemys picta*) hatchlings. *J. Exp. Biol.* **217**, 4174–4183. (doi:10.1242/jeb.111120)
 33. Beck DD. 2005 *The biology of Gila monsters and beaded lizards*. Berkeley, CA: University of California Press.
 34. Davis JR, DeNardo DF. 2010 Seasonal patterns of body condition, hydration state, and activity of Gila monsters (*Heloderma suspectum*) at a Sonoran Desert Site. *J. Herpetol.* **44**, 83–93. (doi:10.1670/08-263.1)
 35. Davis JR, DeNardo DF. 2007 The urinary bladder as a physiological reservoir that moderates dehydration in a large desert lizard, the Gila monster (*Heloderma suspectum*). *J. Exp. Biol.* **210**, 1472–1480. (doi:10.1242/jeb.003061)
 36. Davis JR, DeNardo DF. 2009 Water supplementation affects the behavioral and physiological ecology of Gila monsters (*Heloderma suspectum*) in the Sonoran Desert. *Physiol. Biochem. Zool.* **82**, 739–748. (doi:10.1086/605933)
 37. R Core Team. 2013 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See <http://www.R-project.org/>.
 38. Ewert MA, Wilson DS. 1996 Seasonal variation of embryonic diapause in the striped mud turtle (*Kinosternon baurii*) and general considerations for conservation planning. *Chelonian Conserv. Biol.* **2**, 43–54.
 39. Ultsch GR. 2006 The ecology of overwintering among turtles: where turtles overwinter and its consequences. *Biol. Rev.* **81**, 339–367. (doi:10.1017/S1464793106007032)
 40. Burke RL, Capitano W. 2011 Eastern box turtle, *Terrapene carolina*, neonate overwintering ecology on Long Island, New York. *Chelonian Conserv. Biol.* **10**, 256–259. (doi:10.2744/CCB-0855.1)
 41. Jones MEH, Anderson CL, Hipsley CA, Müller J, Evans SE, Schoch RR. 2013 Integration of molecules and new fossils supports a Triassic origin for Lepidosauria (lizards, snakes, and tuatara). *BMC Evol. Biol.* **13**, 208–228. (doi:10.1186/1471-2148-13-208)
 42. DeNardo DF, Moeller KT, Seward M, Repp R. 2018 Data from: evidence for atypical nest overwintering by hatchling lizards, *Heloderma suspectum*. Dryad Digital Repository. (doi:10.5061/dryad.s1r15c6)